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STRUCTURE OF ESTRIOL GLUCURONIDE FROM HUMAN PREGNANCY URINE

M. Neeman and Y. Hashimoto[1]

Roswell Park Memorial Institute,

Buffalo, New York

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SODIUM estriol glucosiduronate was isolated from human pregnancy urine in 1936 by Cohen and Marrian,[2] but its complete structure remained unknown. We present evidence for the structure of pure estriol glucosiduronic acid isolated in our laboratory.

Twenty liters of third trimester human pregnancy urine was processed by a semi-continuous modification of the extraction sequence developed by Grant and Marrian.[3] However, the first extraction with n-butanol was done at pH 7.5, and the subsequent isolation proved to be more efficient. The

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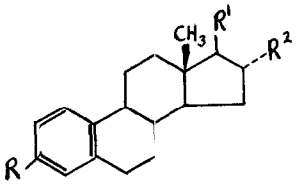
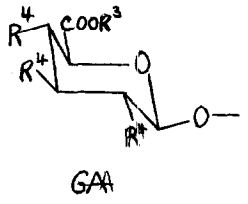
[1] Postdoctoral fellow supported by Institutional Grant to Roswell Park Memorial Institute from the United States Public Health Service.

[2] S.L. Cohen and G.F. Marrian, Biochem. J. 30, 57 (1936). In retrospect it seems probable that the isolated material may have been contaminated with glucuronides of 16-epiestriol, 16α-hydroxyestrone etc., though there is no evidence that this was actually the case (Dr. G.F. Marrian, private communication).

[3] J.K. Grant and G.F. Marrian, Biochem. J. 47, 1 (1950).

urine, in which 10-13 mg of estriol per liter was determined by analysis,[4] afforded 0.286g of crystalline sodium estriol glucosiduronate (I), m.p. 243° (dec.),[5] λ_{max}^{EtOH} 280mμ. Estriol glucosiduronic acid (II), previously described as amorphous,[3] was obtained crystalline for the first time by dissolving the salt I in water saturated with n-butanol and acidifying the solution with hydrochloric acid to pH 1. This procedure was repeated twice, affording analytically pure acid II as tiny white needles, 0.150g of II from 0.275g of I, m.p. 221-222° (dec.), λ_{max}^{EtOH} 280mμ (ε 2100), $\lambda_{max}^{0.05N\ NaOH-EtOH}$ 300mμ (C, 61.79; H, 7.27; neutral equiv. 478).





GAA

Compound	R	R^1	R^2	R^3	R^4
I	OH	OH	GA	Na	OH
II	OH	OH	GA	H	OH
III	OMe	OH	GA	Me	CH
IV	OMe	OMe	GA	Me	OMe
Va≡Vb	OMe	OMe	OH		
VI	OAc	=O	OAc		
VII	OAc	OH	OAc		
VIII	OAc	OMe	OAc		
IX	OH	OMe	OH		

Estriol glucosiduronic acid II, on treatment with diazomethane in dichloromethane - methanol, afforded the methyl ether-ester (III), λ_{max}^{EtOH} 278mμ, 286mμ. The ester III, without purification, was exhaustively

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[4] J.B. Brown, Biochem. J. 60, 185 (1955).

[5] All melting points were determined on a Kofler Audiohm Thermistor hot stage of A.H. Thomas Co., Philadelphia, Pa.

methylated by diazomethane in dichloromethane in the presence of boron trifluoride catalyst.[6] The totally methylated product (IV),[7] obtained in 49.5% overall yield from II, m.p. 140-141°, λ_{max}^{EtOH} 279mμ(ε1800), 287mμ (ε1620), $\lambda_{max}^{CH_2Cl_2}$ 5.72μ, 8.77μ(C, 65.78; H, 8.13), was hydrolyzed by boiling 4 N hydrochloric acid in 10% ethanol to 3,17β-dimethoxy-1,3,5(10)-estratrien-16α-ol (Va),[7] thin plates, m.p. 165-166°, λ_{max}^{EtOH} 279mμ(ε1990), 287mμ(ε1820). This degradation product Va had ultra-violet and infrared spectra superposable with those of the synthetic product Vb of unequivocal structure described below, and the compounds showed an undepressed mixture melting point.

The intermediate in the partial synthesis of estriol,[8] 3,16α-diacetoxy-1,3,5(10)-estratrien-17-one(VI), was reduced with sodium borohydride in methanol below +5° to 3,16α-diacetoxy-1,3,5(10)-estratrien-17β-ol (VII), m.p. 154.5-155.5°, λ_{max}^{EtOH} 268mμ(ε710), 275mμ(ε653), $\lambda_{max}^{CH_2Cl_2}$ 5.69μ, 5.79μ, 8.25μ(broad) (C, 71.12; H, 7.57; acetoxyl, 22.8).[9] Acid catalyzed diazomethane methylation[6] of VII afforded 3,16α-diacetoxy-17β-methoxy-1,3,5(10)-estratriene (VIII), m.p. 108-109°, λ_{max}^{EtOH} 268mμ(ε810), 275mμ(ε780),

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[6] M. Neeman, M.C. Caserio, J.D. Roberts and W.S. Johnson, Tetrahedron 6, 36 (1959).

[7] The compound was chromatographically pure.

[8] N.S. Leeds, D.K. Fukushima and T.F. Gallagher, J. Am. Chem. Soc. 76, 2943 (1954).

[9] A portion of VII was re-oxydized to VI by chromic anhydride-pyridine reagent, G.I. Poos, G.E. Arth, R.E. Beyler and L.H. Sarett, J. Am. Chem. Soc. 75, 422 (1953).

$\lambda_{max}^{CH_2Cl_2}$ 5.68μ, 5.78μ, 8.28μ(broad) (C, 71.30; H, 8.08; methoxyl, 7.85), which was saponified with 10% ethanolic sodium hydroxide to 17β-methoxy-1,3,5(10)-estratrien-3,16α-diol (IX), m.p. 191°, λ_{max}^{EtOH} 281mμ(ε 2010) (C, 75.27; H, 8.69; methoxyl, 10.59). The diol IX was methylated with diazomethane in dichloromethane-methanol to the synthetic 3,17β-dimethoxy-1,3,5(10)-estratrien-16α-ol (Vb),[7] thin plates, m.p. 165-166°, λ_{max}^{EtOH} 279mμ (ε1990), 287mμ(ε1820) (C, 76.03; H, 8.90), identical with the product Va obtained by exhaustive methylation and hydrolysis of estriol glucosiduronic acid II.

We have confirmed the earlier findings of Marrian et al.[2,3] which indicated a partial structure for estriol glucuronide having one alcoholic hydroxyl group combined by a β-glycosidic linkage to a uronic acid, most likely glucuronic acid. On the basis of our evidence we assigned to urinary estriol glucosiduronic acid II, m.p. 221°(dec.), the structure of 3-hydroxy-1,3,5(10)-estratrien-17β-ol-16α-yl-β-D-glucopyranosiduronic acid.

The structures of other glucuronides related to II in human pregnancy urine are being studied.[10]

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